Comparison of the Effects of Chemicals with Combined Perinatal and Adult Exposure vs. Adult only Exposure in Carcinogenesis Bioassays

INTRODUCTION

Based upon their review of animal experimental evidence and human epidemiology data, some investigators have postulated that the age of initial exposure to a chemical carcinogen might influence the carcinogenic response (Vesselinovitch et al. 1979, Rice 1979, McConnell 1992). Some investigators have postulated that perinatal exposure [1] may influence the induction of tumors and have suggested that a perinatal exposure component be incorporated into the current standard carcinogenesis bioassay [2] (Swenberg 1979). In addition, there is an increased public concern about the potential adverse health effects on children exposed to environmental pollutants and pesticide residues in food. Recognizing this concern, U.S. EPA and the International Life Sciences Institute organized a conference in November, 1990, to examine the scientific evidence relative to the broad question of Similarities and Differences Between Children and Adults : Implications for Risk Assessment . At this conference, the results of a literature review regarding carcinogenic responses as a function of age at first exposure were presented (McConnell, 1992). This report concluded that the routine use of a carcinogenesis bioassay with a perinatal exposure component [3] for testing "unknown" chemicals is not warranted to detect a chemical's potential carcinogenic activity.

Subsequently, the National Research Council (NRC) of the National Academy of Sciences (NAS) published a study (1993), *Pesticides in the Diets of Infants and Children* . The NAS recommended that EPA develop toxicity testing procedures that specifically evaluate the vulnerability of infants and children. The newly enacted Food Quality Protection Act (P.L. 104-170) states, "EPA shall assess the risk of the pesticide chemical residues based on available information concerning the special susceptibility of infants and children to the pesticide chemical residues, including neurotoxico- logical differences between infants and

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1. Perinatal exposure : exposure of the maternal animals to the test chemical prior to mating and continued to weaning; the offspring are also exposed to the chemical until 8 weeks old.

2. Standard carcinogenesis study : the test animals are exposed to a chemical for a lifetime beginning at approximately 8 weeks old. This exposure protocol is also referred to as adult exposure in this document.

3. Perinatal carcinogenesis bioassay : A carcinogenesis bioassay that has a perinatal exposure component.

children and adults, and effects of in utero exposure to pesticide chemicals" (SEC. 405)(VI)(C)(II). This Act has further enhanced the importance of examining the value of the perinatal carcinogenesis bioassay. The Health Effects Division (HED) of the Office of Pesticide Programs has examined whether or not a perinatal carcinogenesis bioassay would be of greater value than the standard carcinogenesis bioassay in evaluating the carcinogenic potential of pesticides. Its review and conclusions are presented below.

DISCUSSION

The available carcinogenicity data were reviewed to determine the potential value of a perinatal component in a standard carcinogenesis bioassay. This evaluation consisted of three parts:

(1) analysis of all the studies reviewed and/or referenced by McConnell (1992),
(2) evaluation of the six comparative perinatal carcinogenesis studies conducted by the National Toxicology Program (NTP), and
(3) evaluation of 13 perinatal carcinogenicity studies obtained from the files of the Toxicology Branches of the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration (U.S. FDA).

The following is a summary of this evaluation:

I. <u>Studies reviewed or referenced by McConnell, 1992</u>:

Most of the studies referenced by McConnell were not conducted according to today's accepted procedures for carcinogenicity studies. In addition, most of them were not designed to compare carcinogenic responses following perinatal exposure to those of standard carcinogenesis studies (Table 1; pages 12, 13 and 14). In light of these considerations, it was decided to concentrate on those carcinogenicity studies with a perinatal exposure component even in the absence of parallel carcinogenicity bioassays. The data from the selected studies are presented in Table 2 (pages 15, 16, & 17), and a summary of these studies is provided in Addendum A. Results of these studies indicated that:

. Perinatal exposure seldom produced types of tumors not found in the standard bioassay.
. Exposure of animals to carcinogens beginning during the neonatal period and continuing throughout the animals' lifetime often produced a higher incidence of tumors.
. The latency period to tumor occurrence appeared to decrease in standard carcinogenicity studies with a perinatal exposure component. (This was seen in the studies with vinyl chloride and ETU).

II. <u>NTP studies</u>

The National Toxicology Program (NTP) conducted six carcinogenicity studies using rats and mice with ethylene

thiourea (ETU), polybrominated biphenyls (PBB), and 5,5-diphenylhydantoin (DPH). These three chemicals were selected for this testing because they were known animal carcinogens, able to cross the placenta, and found in the milk of treated dams.

The treatment regimen for these studies contained three components: (i) a standard exposure protocol, (ii) perinatal exposure only, and (iii) a perinatal plus standard exposure protocol. The results of these studies are presented in Table 3 (pages 20 & 21), and a summary of each study is presented in Addendum B. The results indicated that:

. Perinatal exposure alone did not consistently cause an increase in tumor incidence.
. The studies with perinatal exposure alone or adult exposure alone produced similar tumor types in similar tissues.
. Combined perinatal and adult exposure produced a higher tumor incidence relative to that of either the perinatal or adult exposure alone.

III. <u>FDA perinatal carcinogenesis bioassays</u>

Summary data on 19 chemicals, which were tested with the perinatal carcinogenesis study protocol, were identified in the data files of the Toxicology Branches of the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration (FDA). The original reports and/or the data evaluation memoranda for 13 of these chemicals were obtained and evaluated. Additional details of these studies are presented in Addendum C, and the summary of each study is presented in Table 4.

The results of the FDA studies did not show a difference in the ability to detect the carcinogenic potential of a chemical by employing a standard carcinogenesis bioassay or a standard carcinogenesis bioassay with a perinatal exposure component.

CONCLUSION

The observations derived from each part of the evaluation are summarized in Table A.

Table A. Observations Derived From Each Evaluation

Studies Referenced and evaluated by McConnell	NTP Studies	FDA Perinatal Carcinogenesis Bioassays
. Perinatal exposure seldom produced types of tumors not found with the standard bioassay. . Exposure of animals to carcinogens beginning at the neonatal period and continuing throughout the animal's life time produced a slightly higher incidence of tumor. . The latency period to tumor occurrance somtimes appeared to decrease in perinatal carcinogenesis bioassay.	. Perinatal exposure alone did not consistently cause an increase in tumor incidence. . The studies with perinatal alone or adult exposure alone produced similar tumor types in similar tissues. . Combined perinatal and adult exposure produced a slightly higher tumor incidence relative to that of either the perinatal or adult exposure alone.	. The results of the available FDA studies did not show a difference in ability to detect the carcinogenic potential of a chemical by either a standard carcinogenesis bioassay or a perinatal carcinogenesis bioassay.

Of the 69 carcinogenicity studies that were evaluated, 40 studies contained combined perinatal and adult exposure components or at least the pregnant females were exposed to the test chemical during the gestation period. Of the remaining 29 carcinogenicity studies, 12 contained a neonatal exposure component. Among all these studies, the most valuable data were derived from the six NTP studies, the FDA carcinogenicity studies which had a perinatal exposure component, and a series of studies conducted by Vesselinovitch et. al(1979). The following observations are derived from these data:

. Perinatal exposure rarely identifies carcinogens that are not found in standard carcinogenicity studies.
. Combined perinatal and adult exposure slightly increases the incidence of a given type of tumor; it is not known if this reflects the effect of an increased length of exposure or a heightened sensitivity of the young animal to the carcinogenic effects of the chemical.
. The combined perinatal and adult exposure sometimes reduces the latency period for neoplasms to develop.

Therefore, currently available data <u>do not support</u> routinely

incorporating a perinatal exposure component in the standard carcinogenesis bioassay. The available data base for drawing conclusions is not very robust. To address NAS concerns and the Food Quality Protection Act requirement to assess the risk of special susceptibility of infants and children, the Agency will assess and develop criteria for the inclusion of a perinatal exposure component in a carcinogenicity study and apply them on a case-by-case basis. The criteria will include factors such as the likelihood of widespread exposure to women of childbearing age, infants and children and specific toxicity to the developing fetus. This conclusion is consistent with that of U.S. FDA which has developed a set of criteria for determining the candidate for perinatal carcinogenesis bioassay. The criteria are based on use, exposure, and toxicity seen in developmental toxicity and reproduction studies (Addendum C; p. 22)(U.S. FDA, 1982).

ADDENDUM A
Summaries And Conclusions Of Carcinogenicity Results From Selected Studies Also Reviewed by McConnell

Conclusion

This set of data indicated that:

1. The tumor types were similar in carcinogenicity studies with or without perinatal exposure.
2. Initiation of exposure of animals to carcinogens during the neonatal period often produced a higher incidence of tumors than those in animals when exposure began near or after puberty.
3. Perinatal plus adult exposure appeared to decrease the latency period to tumor occurrence; it is not known if it is the result of the increased length of exposure time to the test chemical or a heightened sensitivity of the young animal to exposure of the carcinogen.

Discussion

To evaluate the responses in carcinogenicity studies as a function of age at first exposure, McConnell (1992) conducted a systematic search of various computerized literature data bases such as Cancer Line (1963-1990), Medline (1965-1990), and Toxline (1965-1990). The evaluation conducted by McConnell was mainly restricted to the following parameters:

. cancer endpoints,
. mice, rats, and hamsters,
. studies conducted in the same strain/species,
. studies using a similar route of exposure, and
. studies where the duration of treatment or observation was sufficiently long to assure that a carcinogenic response was possible.

McConnell identified the carcinogenicity studies with a perinatal exposure component and then compared the results of these studies with those of standard bioassays. A summary of the comparison is presented in Table 1, which is excerpted from Comparative Responses in Carcinogenesis Bioassays as a Function of Age at First Exposure (McConnell, 1992). As shown in Addendum A, McConnell analyzed and compared 47 studies for a total of 24 chemicals (treating the 10 nitrosamines as a chemical group). Among these chemicals, 18 showed carcinogenic activity with both perinatal and adult exposure. Aflatoxin, ethylene thiourea, and saccharin showed carcinogenic activity with adult exposure only. Eugenol possibly showed carcinogenic activity with perinatal exposure alone. Based on these data, he concluded the following:

1. Perinatal exposure rarely identifies carcinogens that are not found using a standard bioassay protocol.
2. Perinatal exposure in conjunction with adult exposure usually increases the incidence of a given type of neoplasm.
3. The types of neoplasms found with perinatal exposure are usually also seen with adult exposure.
4. Perinatal exposure in conjunction with adult exposure usually reduces the latency period for developing neoplasms.

The Agency concurs with the McConnell conclusions.

Summary of some individual chemicals presented in Table 2

Aflatoxin B_1:

An increase in the incidence of hemorrhagic liver tumor was seen in rat carcinogenicity studies with an in utero exposure phase as well as with adult exposure (Table 2; Ward et al., 1975).

Metronidazole:

An increase in lung tumor incidence was seen in Swiss mice which were exposed to metronidazole during gestation or adult life. However, no increase in lung tumors was seen in mice which were exposed during gestation, lactation, and adult life. The absence of a carcinogenic effect in a combined perinatal and adult exposure study was attributed by the authors to the toxic dose level of the compound administered (Table 1; Chacko & Bhide, 1986). In an adult exposure study, 6 to 8 weeks old Swiss mice were fed metronidazole in the diet for the life span of the test animals. An increase in the lung tumor incidence was seen in all treated groups (Table 2; Rustia & Shubik, 1972).

Diethylnitrosamine:

With intraperitoneal injection of diethylnitrosamine to C57BL x C3HF1mice at a dose of 10 mg/kg body weight beginning at either 15 or 42 days of age, there was an increase in the incidence of hepatomas was found in males and females of the 15 day-old group and in males of the 42 day-old group. The increase was greater in the 15 day-old group. No increase in hepatomas was seen in the females of the 42 day-old group or in the control males or females. The test animal were sacrificed on 66 weeks of age (Table 2; Rao & Vesselinovitch, 1973).

Diethylstilbestrol (DES):

Subcutaneous injection of DES in female neonates of CD-1 mice from days 1 to 5 postnatally produced an increase in the incidence of uterine tumors in animals sacrificed at 12 or 18 months (Table 1; Newbold, Bullock, & McLachlan, 1990). Following a similar route of administration of DES to pregnant CD-1 mice from gestation days 9 through 16, the female offspring, which were sacrificed at the ages of

12 to 18 months, developed an increased incidence of vaginal, cervical, and uterine tumors in the 10 and 100 µg/kg groups. No increase in tumor incidence was seen in 0.01, 1, 2.5, and 5 µg/kg groups (Table 2; McLachlan, Newbold, and Bullock, 1980).

Benzidine (BZ):

In one of the comparative experiments with B6C3F1 mice, BZ was administered in feed to pregnant females from gestation day 12 to delivery (prenatal exposure), to mothers with litters from delivery to weaning (preweaning exposure), and to offspring from weaning to 90 weeks old (adult exposure). In addition, two combinations of the above exposure schemes (prenatal + preweaning exposure and prenatal + preweaning + adult exposure) were also examined. The results showed that there was an increase in the incidence of liver tumors in both male and female mice regardless of the exposure scheme. However, in male mice, the increased incidence of liver tumor was 62/65 (95%) with the preweaning exposure. A combination exposure scheme containing a preweaning exposure component yielded a liver tumor incidence of 49/49 or 50/50 (100%). In females, the most dramatic increase was seen with the adult exposure (48/50, 96%) or with the exposure scheme including an adult exposure component (47/50, 94%) (Table 2; Vesselinovitch et al., 1979).

Vinyl chloride (VC):

Sprague-Dawley rats were exposed to VC at 6,000 or 10,000 ppm via the inhalation route (4 hours/day) from gestation days 12 to 18 (in utero exposure), from 1 day old (newborn exposure) for 5 weeks, or from 11 weeks old (adult exposure) for 5 weeks. The in utero, newborn, and adult exposure animals were sacrificed at 143, 124, and 165 weeks, respectively. There was no increase in the incidence of liver angiosarcomas or hepatomas in the in utero only exposure groups, and there was an equivocal increase in these two tumor types in 10,000 ppm with the adult exposure. In contrast, in the newborn exposure group, there was a substantial increase in the incidence of liver angiosarcomas and hepatomas (Table 1; Maltoni et al., 1981).

In a comparative experiment, groups of Sprague-Dawley rats were exposed to VC at concentrations of 2500 ppm. One group of females was exposed to VC beginning at 13 weeks of age for a total of 76 weeks (4 hours/day for 7 weeks and then 7 hours/day for 69 weeks) . A second group was exposed from gestation day 12 through delivery, and the offspring continued to be exposed for a total of 15 weeks (4 hours/day for 7 weeks and then 7 hours/day for 8 weeks). A third group was exposed in a similar manner as the second group except the total exposure period was 76 weeks (4 hours/day for 7 weeks and then 7 hours/day for 69 weeks). An untreated control group was also included in the study. An increase in the percentage of animals with hepatocarcinomas, angiosarcomas, and neuroblastomas was seen in all exposure groups. The average latency period to tumor occurrence was decreased in the groups exposed with a combination of in utero and adult exposure scheme with 76 weeks of treatment compared to the group treated for only 15 weeks. In addition, the percent of animals with neuroblastomas was less in the 15 week treatment group relative to that

of animals in the third group (76 weeks of exposure) (Table 2; Maltoni & Cotti, 1988). This may reflect the effects of longer exposure in the animals which were exposed for 76 weeks beginning during gestation.

In a series of comparative studies conducted by Drew et al. (1983), Fischer-344 rats, golden Syrian hamsters, B6C3F1 mice, and CD-1 Swiss mice received VC by the inhalation route beginning at different ages for various durations. The exposure concentrations were 100 ppm for rats, 50 ppm for mice, 200 ppm for hamsters. For comparative purposes, the results from the groups which were exposed for 12 months beginning at approximately 5 weeks or 6 months old are excerpted from the report and summarized in Table 2. Increases in the incidence of hemangiosarcomas, mammary gland tumors, and hepatocellular carcinomas were found in rats, mice, and hamsters. The incidences were substantially greater when these test animals were exposed at the younger ages (Table 2; Drew et al., 1983). The results of this experiment and those of Maltoni et al. (1981) are presented to illustrate the point that exposure of very young animals (less than 5 weeks old) to certain chemicals can result in an increase in the tumor incidence.

Table 1[+]
Comparative Carcinogenic Response as a Function of Age[a] During Exposure

Chemical and Its Genotoxicity	Species	Route (and Age)[a]	Results	Source
Afiatoxin (+)[b]	Rat	Feed G(1-12d) G+1(to 2w)	 No neoplasms No neoplasms	Grice et al. (1973)
	Rat	Feed G+1+A(8-104 w)	 Liver and colon	Ward et al. (1975)
Anethole(-)	Mouse	Inject/feed I(1,8,15 and 22d) A(8-52)	 Liver Liver and vascular	Miller et al. (1983)
Asbestos (chrysotile) (+)	Rat	Feed G+1+A	 Colon (low incidence)	NTP (1985)
	Rat	Feed A	 Colon (low incidence)	Donham et al. (1980)
B(a)P(+)	Mouse	Inject G(11,13, and 15)	 Lung	Bulay and Wattenberg (1970
	Mouse	Feed A(17-197 d)	 Forestomach	Neal and Rigdon (1967)
Benzidine (+)	Mouse	Gavage/Feed G(12+d) G+I A(6-9w) A(4-90w) G+1	 Liver (low indidence, male only) Liver (high incidence, male only) No response Liver (high) incidence, male and female), lung and Harderian gland	Vesselinovitch (1975, 1979)
(Cycasin (+)	Rat	Feed G(1,2 or 3w)	 Various sarcomas	Spatz and Laqueur (1967)
	Rat	Inject I(0,7,14, or 21 d)	 Kidney, liver, colon (higher incidence on D 0 and 7 than on D 14, none on day 21)	Fukunishi et al. (1985)
	Rat	Oral A (multiple)	 Colon, kidney, and liver	Laqueur et al (1981)

Table 1[+]
Comparative Carcinogenic Response as a Function of Age[a] During Exposure

Chemical and Its Genotoxicity	Species	Route (and Age)[a]	Results	Source
DEN (+)	Mouse	Inject I(15 d) A(42 d)	 Liver (high incidence, male and female) Liver (low incidence, male only)	Rao and Vesselinovithc (1973) Vesselinovitch et al. (1979)
	Mouse	Inject I(1 or 15d)	 Liver (higher incidence at 15D)	Druckrey (1973)
	Rat	Inject Oral	 Olfactory, liver, kidney Same, but higher incidence in liver and kidney	
DES(-)	Mouse	Inject G (9 or 16 d) Inject I(1-5 d) Feed A(6-18 m) Feed A(6-136 w)	 Vagina, cervix, and uterus Uterus Mammary gland Cervix and uterus	McLachlan et al. (1980 Newbold et al. (1980) Glass et al. (1974) Highman et al. (1977)
ENU(+)	Mouse	Inject G(12,14,16 or 18 d) I(1 or 15 d) A(42 d))	 Kidney Kidney (similar incidence) Kidney (lower incidence)	Rice (1979)
	Mouse	Inject G(12,14,16 or 18 d) I(1,15 or 42 d)	 6 organs (higher incidence at 16 and 18 d) 22 organs	Vesselinovitch et al. (1979)
	Rat	Inject G(10-23 d) I(0-30 d) A (>30 d)	 Brain and PNS (very high incidence) Same tumors (high incidence) Very low incidence	Rajewsky (1985)
10 nitrosamines (+)	Hamster	Inject G (8,10,12, or 14 d)	Various types (higher incidence and shorter latent period in dams, more tumors in F_1 if exposed later in G)	Althoff and Grandjean (1979)
DDT(-)	Mouse	Gavage/feed I(1-4 w) A(5-90 w) I+A(1-90 w)	 Liver Liver (higher than I) Liver (similar to A)	Vesselinobitch et al. (1979)

Table 1[+]
Comparative Carcinogenic Response as a Function of Age[a] During Exposure

Chemical and Its Genotoxicity	Species	Route (and Age)[a]	Results	Source
Dieldrin (-)	Mouse	Gavage/feed I(1-4 w) A (5-90 w) I+A (1-90 w)	 Liver Liver (higher than I) Liver (higher than A alone)	Vesselinovitch et al. (1979)
DMBA(+)	Mouse	Inject G (14 d)	 Mammary gland, ovary, lung, and lymphoma (F_2 mice also had similar neoplasms)	Tomatis (1965)
	Mouse	Inject G(11,13, and 15 d)	Lung and ovary	Bulay and Wattenert (1970)
	Mouse	Skin paint A (single)	 Papillomas and carcinomas (low incidence)	Ward et al. (1988)
Estrgole (-)	Mouse	Inject/feed I(1,8,15, and 22 d) A (8-52 w)	 Liver Liver and vascular	Miller et al. (1983)
ETU (-)	Rat	Feed G+I (8 w) A (8-104 w) G+I+A	 No tumors Thyroid Thyroid (slightly higher Incidence and shorter latency)	NTP (1990)
	Mouse	G+I (8 w) A(8-104 w) G+I+A	No tumors Thyroid, liver Throid, liver (slightly higher incidence and shorter latency)	
Engenol (-)	Mouse	Inject/feed I(1,8,15, and 22 d) A (8-52 w)	 Liver No Tumors	Miller et al. (1983)
	Mouse	Feed A (6-110 w)	 Liver (equivocal increase)	NTP (1983)
Metronidazole (+)	Mouse	Intragastric G (entire) A (2-22 m) Feed A (entire)	 Lung, liver, thymus (males only) No tumors Lung and lymphoma (males only)	Chacko and Bihde (1986) Rustia and Shubik (1972)

Table 1[+]
Comparative Carcinogenic Response as a Function of Age[a] During Exposure

Chemical and Its Genotoxicity	Species	Route (and Age)[a]	Results	Source
Quinoline (+)	Mouse	Inject/feed		LaVole et al. (1987)
		I(1,8, and 15 d)	Liver	
	Mouse	Feed	Liver	Shinahara et al. (1977)
		A (A-104 w)		
Saccharin (-)	Rat	Feed		Schoenig et al. (1985)
		G (entire)	No tumors	
		I+A	Urinary bladder	
		2 generation	Urinary bladder (no increase)	
Safrole (-)	Mouse	Inject/feed		McLchlan et al. (1980)
		I(1,8,15, and 22 d)	Liver	
		A (8-52 w)	Liver, vascular	
	Mouse	Inject		Vesselinovitch et al. (1979)
		G(12,14,16, or 18 d)	Liver, male only, low incidence	
		I (via dam)	Liver, male only (4x more than G)	
		G+I	Liver, same as I	
		A (44-90 w)	Liver, male and female	
		G+I=A	Liver, male andd female, higher incidence than A	
TCDD (-)	Mouse	Inject		Della Porta et al. (1987)
		I(1x/w.5 w)	Liver, thymus	
		A(1x/w,6-46 w)	Liver	
	Mouse	Gavage		NTP (1982)
		A (8-104 w)	Liver and thyroid	
Urethan (+)	Mouse	Inject		Klein (1952)
		G	Lung, liver, mammary gland	
		G+A	Same organs (higher incidence)	Lansen (1947)

Table 1[+]
Comparative Carcinogenic Response as a Function of Age[a] During Exposure

Chemical and Its Genotoxicity	Species	Route (and Age)[a]	Results	Source
VC (+)	Rat	Inhalation		Laib et al. (1985)
		G (entire)	No liver foci	
		I+A (0,7, 94 21 d +83 d)	Foci (increasing incidence to 17 d, then no increase	
	Rat	Inhalation		
		G (12-18 d)	Zymbal gland	Maltoni et al. (1981), Maltoni and Lotti (1988)
		A (52 w)	Zymbal gland, kidney, liver, angiosacroma, skin, brain, and mammary gland)	
	Rat	Inhalation		Drew (1983)
	mouse, hamster	A (various periods of exposure)	Lung, liver, vascular (higher incidence at younger exposure	

a: Age: G = gestation (exposed during pregnancy), I = infant (lactation to 6 weeks), and A = adult (> 6 weeks); d = day, w = week, m = month.
b: A plus sign means the compound is genotoxic; a minus sign means the compound is not genotoxic.
+: This table is excerpted from Comparative Responses in Carcinogenesis Bioassay as a function of Age at first Exposure (McConnell, 1992); p. 70-72.

Table 2. Selective Studies Previously Reviewed by McConnell

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
Aflatoxin B_1	Carcinog.	1975	F344 rats	Dietary feeding: 2 ppm A). from G. day 1, till death of the offsprings 26 ♂ & 21 ♀	hemorrhagic liver tumors & colon tumor were seen in treated ♂s & ♀s. Colon tumor incidence: 2/20 ♂; 5/14 ♀.	Ward et al.. JNCI 55(1): 107-113, July 1975.
				B). from 6-7 weeks of age till death.	hemorrhagic liver tumors and colon tumors were seen in treated ♂s and ♀s. Colon tumor incidence: 2/5 ♂; 3/14 ♀.	
				C). Controls were fed regular diet (Wayne meal diet)	No tumor incidence was reported for the controls.	
Metronidazole	Carcinog.	1986	Swiss mice	Adult exposure: 2 mg/mouse by gavage on alternate weeks for 25 months.	Marginal increase in the incidence of lung tumors in ♂ mice (treated, 3/40; control 0/40).	Chacko & Bhide. J Cancer Res Clin Oncol 112: 135-140, 1986.
				Perinat. expo .: 2 mg/mouse Group A: during gestation (F_1a)	Increased lung tumor incidence in treated males (treated, 8/40; control, 1/24)	
				Group B: during gestation, (F_1b) lactation, & adult (24 months)	No increase in tumor incidence	
	Carcinog.	1972	Swiss mice 6-8 wks old	Feeding: Dietary concen. 0, 0.06%, 0.15%, 0.3%, & 0.5%. Mice were treated until spontaneous death	Increased incidence of lung tumor was seen in all treated males and females relative to the controls. An increased incidence of malignant lymphoma was also seen in the two high dose group ♀s (0.5%, 18/36; 0.3%, 10/20; Control, 22/70).	Rustia & Shubik. J. Nat Cancer Inst.: 48: 721-729, 1972
Diethylnitrosamine (1973)	Carcinog.	1973	C57BL x C3HF1 mice	Injection (i.p.): ♂ & ♀ received the test chemical (10 mg/kg bw) at 15 or 42 days old. The test animals were sac. at 66 weeks of age	Age(days) / Sex / Hepatomas Incidence: 15 M 25/25 (100%); 15 F 22/25 (88%); 42 M 6/26 (23%); 42 F 0/20 (0%); 15 (V.Cont.) M 0/25 (0%); 15 (V.Cont.) F 0/25 (0%)	Rao, K. N. & Vesselinovitch, S. D. Cancer Research 33:1625-1627. V. Cont. = Vehicle Control

♂: male ♀: female ↑: increase ↓: decrease

Table 2. (Continued)

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
Diethylstilbestrol (DES)	Carcinog.	1990	CD-1 ♀ mice Neonates (Days 1-5 in life)	S.C. injection: Control (corn oil), 0.002 to 2.0 µg/mouse/day from days 1 -5 in life. The treated mice were sacrificed at 1, 2, 4, 6, 8, 12, & 18 months.	The 2 µg/mouse group females which were sacrificed at 12 & 18 months developed uterine adenocarcinomas (12 month, 8/17; 18 month, 9/10; Controls, 0/17 & 0/10 for 12 & 18 month sac., respectively).	Newbold, Bullock, & McLachlan. Cancer Res. 50: 7677-7681, 1990
	Carcinog.	1980	CD-1 ♀ mice pregnant	Pregnant ♀ mice were treated (s.c.) with DES on gestation days 9 to 16 at doses from 0.1 to 100 µg/kg. After delivery litter sizes were adjusted to be 8. The ♀ offspring were sacrificed at 12 to 18 months.	An increase in the incidence of vaginal, cervical, and uterine tumors was seen in 10 and 100 ug/kg group ♀s which were exposed to DES in utero.	McLachlan, Newbold, & Bullock. Cancer Res. 40: 3988-3999, Nov 1980.
Benzidine (BZ)	Carcinog.	1979	B6C3F1 mice	Dietary feeding: 150 ppm BH was fed to pregnant ♀ from day 12 of gestation to delivery (prenatal expos), to mothers with litters from delivery to weaning (preweaning expo), and to offspring from weaning to 90 weeks (adult expos.). Combinations of prenatal+preweaning & prenatal+preweaning+weaning+adult exposures were conducted.	Liver Tumor **Males** Incidence Percent Controls 1/98 1% prenatal (Pren) 17/55 31% Preweaning (Prew) 62/65 95% Adult (Adl) 25/44 59% Pren + Prew 49/49 100% Pren + Prew + Adl 50/50 100% **Females** Controls 0/100 0% Pren 2/62 3% Prew 2/42 5% Adl 48/50 96% Pren + Prew 12/48 25% Pren + Prew + Adl 47/50 94%	Vesselinovitch et al.. Natl. Cancer Inst. Monogr. 51: 239-250, 1979
Vinyl chloride (VC)	Carcinog.	1981	Sprague Dawley rats	Inhalation: 4 hr/day, in utero expo. gest. days 12 to 18. Offspring sac. at 143 wks. Newborn expo. 1 day old, exposed for 5 wks & sac. at 124 wks. Adult expo. 11 wks old exposed for 5 weeks & sac. at 165 weeks. All the test animals were exposed to 6000 or 10,000 ppm VC	% of Animals With Tumors LAS Hepatomas **In utero expo.** 6,000 ppm - - 10,000 ppm - - **Newborn expo.** 6,000 ppm 41 48 10,000 ppm 34 45 **Adult expo.** 6,000 ppm - - 10,000 ppm 1	Maltoni et al..Environ. Health Persp. 41: 3-29, 1981 LAS = Liver angiosarcomas

Table 2. (Continued)

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
	Carcinog.	1988	Sprague Dawley rats	Inhalation: Adult (13 wks old) ♀s were exposed for 4-7 hr/d, 5 d/wk for 76 wks. In utero (12th day of gestation) exposure for 4-7 hr/d, 5 d/wk for 76 or 15 weeks.	% of Animals With Tumors Hpt / Ang / Neubl **Adult** (76wk expo.)(♀) 2500 ppm 9 (63) / 50 (60) / 59 (53) 0 ppm - / - / - **In utero** (76 wks expo.) 2500 ppm ♂ 42 (54) / 56 (53) / 48 (47) ♀ 60 (51) / 73 (51) / 43 (48) **In utero** (15 wk expos.) 2500 ppm ♂ 70 (84) / 40 (78) / 12 (61) ♀ 72 (81) / 47 (74) / 18 (54) **In utero Controls** 0 ppm ♂ 1 (102) / - / - ♀ - / - / -	Maltoni & Cotti (1988) Annals NY Academy of Sci. 534:145-159. Hpt= hepatocarcinomas Ang= angiosarcomas Neubl= neuroblastomas ( ) = average latency (wks)
	Carcinog.	1983	B6C3F1 ♀ mice, CD-1 Swiss ♀ mice, Fischer-344 ♀ rats & golden Syrian hamsters	Inhalation: rats, 100 ppm; mice, 50 ppm; and hamsters, 200 ppm. 5 weeks to 12 months old animals were exposed 6 hr/d, 5 d/wk to VC for 12 months.	Exp. Mos / Hmgsa / Magltu / Heptcarci Rats 0-12: 11/55** / 11/56** / 4/56** 6-18: 5/54** / 4/55 / 1/54 Cont.: 1/112 / 5/112 / 1/112 CD-1 Swiss mice / Lung carc 0-12: 30/47** / 22/47** / 15/47** 6-18: 17/46** / 8/45** / 9/46* Cont.: 1/71 / 2/71 / 9/71 B6C3F1 mice 0-12: 69/90** / 37/90** 6-18: 30/48** / 9/48** Cont.: 4/69 / 3/69 Hamsters 0-12: 4/52** / 31/52** 6-18: 1/44 / 6/44** Cont.: 0/143 / 0/143	Drew, R. T. et al. (1983) Tox. & Applied Pharmacol. 68: 120-130 Hmgsa = hemangiosarcomas Magltu = mammary gland tumors (carcinomas in hamsters & mice; adenocarcinomas in rats) Lung carc = lung carcinomas Heptcarci= hepatocellular carcinomas

*: Different from controls p<0.05 (life table analysis)
**: Different from controls p<0.01 (life table analysis)

ADDENDUM B

Summaries And Conclusions Of The NTP Studies

Conclusions

The six NTP studies were designed specifically to compare the carcinogenic response of the three chemicals (ethylene thiourea [ETU] , polybrominated biphenols [PBB], & 5,5-diphenylhydentoin [DPH]) which were administered to rats and mice with perinatal exposure alone, adult exposure alone, and combined perinatal and adult exposure (Table 3). The results showed that:

1. The perinatal exposure alone did not consistently cause an increase in tumor incidence.
2. The studies with the perinatal exposure alone or the adult exposure alone produced similar tumor types in similar tissues.
3. The combined perinatal and adult exposure produced a higher tumor incidence relative to that of either the perinatal or adult exposure alone.

Summaries of the individual chemicals (Table 3)

For **ETU**, the perinatal-only exposure produced no carcinogenic effects in either rats or mice. Adult exposure induced thyroid follicular cell tumors in rats and mice and hepatocellular and pituitary gland tumors in mice. The combined perinatal and adult exposure confirmed what was seen in the adult-only exposure study with a slightly higher incidence of thyroid follicular cell tumors in rats. In general, the carcinogenic effects of ETU were not influenced by the incorporation of perinatal exposure into the conventional adult exposure study for 2 years in rats and mice (Chhabra et al., 1992).

For **PBB**, the perinatal-only exposure marginally increased the incidence of hepatocellular tumors in male rats only, while in mice perinatal-only exposure produced hepatocellular tumors in both sexes. Adult-only exposure induced

hepatocellular tumors in both sexes of rats and mice. The combined adult and perinatal exposure confirmed the increased hepatocellular tumor rate in rats and mice. The data also showed that combined perinatal and adult exposure increased PBB-related hepatocellular tumors in comparison to the adult-only exposure. An increased incidence of mononuclear cell leukemia in both male and female rats was found in perinatal-only exposure, adult-only exposure, and combined perinatal and adult exposure to PBB (Chhabra et al., 1993).

For **DPH**, perinatal-only exposure did not increase tumor incidences at any sites in rats, but it produced a marginal increase in hepatocellular tumors in female mice. Adult-only exposure induced an increase in hepatocellular tumors in female mice and a marginal increase in this tumor in male rats. The combined perinatal and adult exposure produced an increase in hepatocellular tumors in male rats and in male and female mice. In addition, the combined perinatal and adult exposure yielded an increase in the incidence of hepatocellular tumors in male mice that was not seen in adult-only exposure (Chhabra et al., 1993a).

Table 3. NTP studies which have perinatal and adult exposures

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
Ethylene thiourea (ETU)	Oncogenicity perinatal, perinatal + adult, & adult expo-only.	1991	F344/N rats	Perinatal exposure: 0, 9, 30, & 90 ppm in diet Adult exposure: 0, 25, 83, & 250 ppm	Perinatal exposure only produced no carcinogenic effect in either sexes. Adult exposure induced thyroid follicular cell tumors. Perinatal and adult exposure also produced thyroid follicular cell tumors, and the incidence was slightly higher than that with adult only exposure.	Chhabra et al. 1992
	Oncogenicity perinatal, perinatal + adult, & adult expo-only	1991	B6C3F1 mice	Perinatal exposure: 0, 33, 110, & 330 ppm in diet Adult exposure: 0, 330, or 1000 ppm	Perinatal exposure only produced no carcinogenic effect in either sexes. Adult exposure induced thyroid follicular cell tumors, hepatocellular adenomas or or carcinomas, and pituitary gland tumor. Perinatal and adult exposure also produced thyroid follicular cell tumors, hepato-cellular adenomas or carcinomas, and pituitary gland tumor.	Chhabra et al. 1992
Polybrominated biphenyls (PBB)	Onco. peri-natal expo., perinatal + adult expo., adult expo. only	1993	F344/N	Perinatal exposure: 10 ppm in the diet. Adult exposure: 0, 10, & 30 ppm in the diet Perinatal + adult exposures (F_0:F_1): 1:3, 3:10, 10:10, 10:30 ppm in the diet	Perinatal exposure alone to PBB produced a marginally increased incidence of hepa-tocellular adenomas in 10 ppm male rats. Adult exposure produced increased incidence of hepatocellular neoplasms in both sexes. Combined perinatal and adult exposure also produced hepatocellular neoplasms. The perinatal exposure enhanced the suscepti-bility of female rats receiving adult exposure in producing liver tumors. An increased incidence of mononuclear cell leukemia in both male and female rats was also associated with perinatal, adult only, & combined perinatal & adult exposures to PBB.	Chhabra et al. 1993
	Onco. peri-natal expo., perinatal + adult expo., adult expo. only	1993	B6C3F1 mice	Perinatal exposure: 30 ppm in the diet. Adult exposure: 0, 10, & 30 ppm in the diet Perinatal + adult exposures (F_0:F_1): 3:3, 10:10, 30:10, 30:30 ppm in the diet	Perinatal exposure alone to PBB produced increased incidence of hepatocellular neoplasm in both sexes of mice. Adult exposure produced increased incidence of hepatocellular neoplasms in both sexes. Combined perinatal and adult exposure also produced hepatocellular neoplasms, and the combined exposure appeared to increase the treatment-related increase in hepato-cellular neoplasms relative to the adult only exposure.	Chhabra et al. 1993

Table 3. (Continued)

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	Sources
5,5-Diphenylhydantoin (DPH)	Onco. perinatal expo., adult expo., perinatal + adult expo.	1993	F344/N rats	Perinatal exposure: 630 in the diet Adult expo.: 0, 800, & 2400 ppm in the diet Perinatal + adult expo. (F0:F1): 63:240, 210:800 630:800, & 630:2400 ppm in the diet	Incidence of hepato. adeno. or carcino. (F_0:F_1)ppm — Male — Female Perinatal exposure group 630:0 — 1/50 — 0/49 Adult expoure group 0:0 — 0/50 — 0/50 0:800 — 2/50 — 1/50 0:2400 — 4/50 — 1/50 Perinatal + adult exposure group 63:240 — 3/49 — 0/50 210:800 — 2/49 — 1/50 630:800 — 1/49 — 0/50 630:2400 — 5/49* — 0/50	Chhabra et al. 1993a *: p<0.05 compared to 0:0 group Historical control Male: 6/302 (2%); range 0-10% Female: 0/300 (0%)
	Onco. perinatal expo., perinatal + adult expo., adult expo. only	1993	B6C3F1 mice	Perinatal exposure:210 ppm in the diet. Adult exposure: M: 0, 100, 300 ppm. F: 0, 200, & 600 ppm in the diet Perinatal + adult exposure (F_0:F_1): M: 21:30, 70:100, 210:100, & 210:300 ppm. F: 21:60, 70:200, 210:200, & 210:600 ppm the in diet	Incidence of hepato. adeno. or carcino. (F_0:F_1)ppm — Male — Female Adult exposure group 0:0 — 29/50 — 5/48 0:100(200) — 29/49 — 14/49* 0:500(600) — 26/49 — 30/50** Perinatal + adult exposure group 21:30(60) — 25/50 — 13/50* 70:100(200) — 31/50 — 26/50** 210:100(200) — 35/49 — 16/50** 210:300(600) — 41/50* — 34/50** Perinatal exposure group 210:0 — 33/50 — 12/49	Chhabra et al. 1993a ( ): ppm for F_1 females * : p<0.05 compared to 0:0 group ** : p<0.001 compared to 0:0 group Historial controls Males — Females 167/410 (40%) — 56/416 (13%) Range — Range 17-68% — 3-26%

ADDENDUM C
FDA Perinatal Carcinogenesis Bioassays

Conclusions

No difference in carcinogenic response for the 13 chemicals was detected when they were tested in either a standard carcinogenicity study or a perinatal carcinogenicity study.

Discussion

In addition to the NTP studies, summary data on 19 chemicals which were tested in chronic/carcinogenicity studies including an in utero exposure phase were obtained from the Toxicology Branches of the Center for Food Safety and Applied Nutrition, U.S. FDA. Some of these studies were conducted in the 1970's or earlier. The original reports and/or the data evaluation memoranda for 13 of the 19 chemicals were obtained and evaluated. The data summary of each study is presented in Table 4. The criteria employed by the Toxicology Branches of the Center for Food Safety and Applied Nutrition, U.S. FDA, for considering chemicals as candidates for testing in a perinatal carcinogenicity study are:

1. Compounds whose lowest "effect" level is less than 200-times the expected human exposure.
2. Compounds which are used as non-nutritive additives and whose exposure exceeds 0.25 mg/kg/day.
3. Compounds which are considered as nutritive additives.
4. Compounds with reproductive toxicity or teratogenic activity.
5. Any compound with data indicating differences in affected organs in in utero studies vs. non-in utero studies which require further study.
6. Compounds with other data (reproductive and developmental toxicity) indicating a need for in utero exposure.

The 13 studies obtained from FDA were conducted slightly differently from the six NTP studies. Both parental animals (rats mainly) were treated prior to and at mating. Treatment of the dams continued through the gestation and lactation periods. At weaning, the dams were sacrificed. The offspring were then exposed continuously to 2 years of age. No carcinogenic response was detected for 12 of the 13 chemicals tested in the standard carcinogenesis bioassay or the perinatal carcinogenesis bioassay. The highest level tested in these studies appeared to be sufficiently high because most of the top dose was approximately 2% of the test diet.

For **sodium nitrite**, a marginal increase in the incidence of lymphoreticular tumor in the spleen was seen in treated rats in both the standard carcinogenicity study and the perinatal carcinogenesis bioassay.

Table 4. Evaluated Studies obtained from FDA

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
FD & C Blue No.2	Chron/onco in utero	1981	CD^R rats	0, 0.5. 1.0, and 2.0% in the diet. The surviving rats for the carcinog. study were sac. at 30 months.	Treatment-related increase in tumor incidence was not seen.	Hogan and Knezevich, 1981 CAP 8C0064 9:1970
FD & C Yellow No.5	Chron/onco in utero	1982	CD^R rats	0.1, 1.0, & 2.0% in the diet	No increase in tumor incidence in treated rats or controls of both phases of the the study.	Sinkeldam, Kuper, & Beems, 1979. CAP5C0023 21:4609, 28:6605
FD & C Yellow No.6	Chron/onco in utero	1982	Sprague Dawley rats	0.75%, 1.5%, & 3.0% in the diet	Tumor incidence in treated rats was comparable to that of the controls in either in utero phase or chronic phase of the study. There was a decrease in pup viability in 3.0% group.	Hogan and Knezevich, 1982 CAP 860066 8:1934
FD & C Yellow No. 6	Chron/onco in utero	1983	CD^R rats	Feeding: 0.0% & 5% for 2 months prior to mating & for 26 to 28 months for the offsprings.	↓ body weights in dosed males and females. No compound-related increase in tumors was seen. ↑ absolute& relative kidney weights in treated ♀s.	Hogan and Knezevich, 1983 CAP 8C0066 19:5501-5767
Modified poly-dextrose	Onco/ in utero	1977	CD-COBS rats	5%, & 10% in diet	No tumor increase in treated animals relative to the controls.	Reinert, 1975 FAP 9A3441 5-6:872-1272
Gellam gum	Chron/onco in utero	1984	Sprague Dawley rats	0; 25,000; 38,000 & 50,000 ppm in diet	Tumor incidence in the treated rats was comparable between treated and control rats.	Batham & Kangas, 1985 FAP 6A3903 7:1761-1844
Acesulfam potassium	Chron/onco in utero	1979	CPB-WU rats	0%, 0.3%, 1.0%, & 3.0% in diet; 60/ sex/dose	No compound related increase in tumor incidence. ↓ body weight and food efficiency in high dose ♂ & ♀. Slight ↓ in hemoglobin in high dose ♀ at weeks 102 & 119 weeks of the study.	Sinkeldam et al., 1977 FAP2A3659 5:001058-001228
FD & C No. 40 (Allura™ Red)	Chron/onco in utero	1977	CD-1 rats (Sprague Dawley)	0%, 0.37%, 1.39%, & 5.19% in diet. F0 animals were exposed 1 week prior to mating. 50/sex/dose	A decrease body weights was seen in high dose males and females. No increase in tumor incidence was seen.	Serota et al., 1977 CMF000017 11:2492-2710
Sodium nitrite	Chron/onco in utero	1978	CD^R Sprague Dawley	0, 250, 500, & 2000 ppm in the diet. 1000 and 2000 ppm in H_2O. Urethane was used as a positive cont., 2000 ppm. Both parental and offsprings animals were exposed for life.	Sodium nitrite caused a marginal increase in lymphoreticular tumors in the spleen at 1000 and 2000 ppm in parental & offspring rats. The spleen of the affected animals was hyperplastic (immunoblastic cell proliferation).	Newberne, 1978 SBJ 001255 18:4692

Table 4 (Continued)

Chemical Name	Study Type	Year	Species/Strains	Route/dose levels	Effects	References
Fumaric acid	Chronic/ in utero	1946	Guinea pigs	1% fumaric acid in diet given twice/week prior to mating.	Animals were reported to do very well, & increased tumor incidence was not found.	Levey et al., 1946 FAP 3A1073 2:486-492
Acetylated monoglycerides	Chronic/ in utero	1958	Slonaker strain rats	10♂ & 5♀ were exposed to 10% acetylated glycerides in diet for 3 months prior to mating. Offspring were fed 5, 10, or 20% acetylated glycerides in diet 2 yrs.	No increase in tumor incidence was seen. "Rusty" discoloration of the uteri was found in half of the treated females.	Ambrose et al., 1985 FAP 0A0059 2:433-455
Malto (3-hydroxyl-4-pyrone)	Chron/onco in utero	1978	Crl:CD-COBS (SD) Rats	0, 100, 200, & 400 mg/kg. F0-20/sex/dose Onco. 50/sex/dose F1 &F2 F0-exposed 70 days prior to mating.	No increase in tumor incidence. No effect on survival. Increased in K^+ level and blood urea nitrogen.	Perrand et al., 1978 FASP 00848 2:341-744
Caramel (4-methyl-imidazole)	Chron/onco in utero	1975	Wistar rats	Feeding: Single strength: 5, 10, or 15 % in diet. Double strength: 2, 4, 16% in diet.	No increase in tumor incidence. A sporadic decrease in body weight was seen.	Sinkeldam et al., 1975 GRP 3T0113 22:6442-6476

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